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(54) Title: USE OF TRANSGLUTAMINASE MODULATORS TO PROMOTE WOUND HEALING

(57) Abstract

There is disclosed inhibitors of transglutaminase for use in promoting the healing of wounds and fibrotic disorders, in particular for promoting the healing of wound and fibrotic disorders with reduced scarring, together with methods for same. Also provided are stimulators of transglutaminase for use in promoting the healing of chronic wounds, together with methods for same.

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USE OF TRANSGLUTAMINASE MODULATORS TO PROMOTE WOUND HEALING

The present invention concerns inhibitors of transglutaminase for use in promoting the healing of wounds and fibrotic disorders, in particular for promoting the healing of wound and fibrotic disorders with reduce scarring, together with methods for same. Also provided are stimulators of transglutaminase for use in promoting the healing of chronic wounds, together with methods for same.

By "wounds" and "fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of eye wounds, including wounds to the cornea, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and proliferative vitreoretinopathy.

In particular, there is a lack of compositions for promoting the healing of wounds or fibrotic disorders with reduced scarring. Scar tissue formation, although providing mechanical strength to a healed wound, can be unsightly and may impair the function of the tissue.

This is particularly the case in wounds which result in scar tissue formation

in the CNS, the scar tissue inhibiting the reconnection of severed or re-growing nerve ends, so significantly affecting their function.

There is also a lack of compositions for use in the treatment of chronic wounds, for example venous ulcers, diabetic ulcers and bed sores (decubitus ulcers), especially in the elderly and wheel chair bound patients. Such compositions may be extremely useful in patients where wound healing is either slow or in whom the wound healing process has not yet started. Such compositions may be used to "kick-start" wound healing and may then be used in combination with compositions (e.g. those of PCT/GB93/00586) which promote the healing of wounds or fibrotic disorders with reduced scarring. Hence not only may a chronic wound be healed, but it may be healed with reduced scarring.

The activation of LTGF-β (Latent TGF-β) to active TGF-β is a critical step in the wound healing process. LTGF-β [which comprises TGF-β bound to the LAP (Latency Associated Peptide) which in turn may be bound to the LTBP (LTGF-β Binding Protein)] binds to cell-surface M6P receptors *via* mannose-6-phosphate (M6P)-containing carbohydrates in the LAP. This binding allows the activation of the LTGF-β. Transglutaminase is important in this activation by cross-linking the LTGF-β to the cell surface and cross-linking cell-surface plasminogen which is activated to plasmin (see Kojima, S., *et al.*, 1993, Journal of Cell Biology, 121(2): 439-448, and references therein). Published research has so far indicated that a general activation of TGF-β (the TGF-β family as a whole) is achieved by transglutaminase. However, the present inventor has found that, surprisingly, a differential activation of fibrotic and non-fibrotic TGF-β may be achieved *via* the use of transglutaminase at the wound site. This has

allowed the preparation of compositions which may be used to differentially inactivate and activate fibrotic TGF-β relative to non-fibrotic TGF-β and therefore either promote the healing of wounds or fibrotic disorders with reduced scarring or promote the healing of chronic wounds respectively. Included as fibrotic TGF-βs are TGF-β_1 and TGF-β_2. Non-fibrotic TGF-βs include TGF-β_3. By "inactivate fibrotic TGF-β" is meant that the fibrotic TGF-β is not converted from its inactive form to its active form.

According to the present invention there is provided an inhibitor of transglutaminase for use in promoting the healing of wounds or fibrotic disorders with reduced scarring. The transglutaminase may be type II transglutaminase.

Inhibition of transglutaminases is well known (for example see US 4968713, EP 0465343, CAPLUS 1987: 642618, CAPLU 1980: 453964 and CAPLUS 1978: 11937). However, the ability of inhibitors of type II transglutaminase to promote the healing of wounds or fibrotic disorders with reduced scarring has neither been suggested nor disclosed.

The inhibitor may be an active site inhibitor or a substrate competitive inhibitor.

The inhibitor may, for example, be cystamine, monodansylcadaverine (MDC), putrescine, bacitracin, 2-[3-(diallylamino)propionyl]benzothiophene, a neutralising antibody or an antigen binding fragment thereof specific to transglutaminase and a derivative of 2[(2-oxopropyl) thio] imidazolium. Cystamine, MDC and putrescine are inhibitors of type II transglutaminase.

A neutralising antibody may be an IgG antibody specific to transglutaminase. A neutralising antibody may for example be a monoclonal , polyclonal or genetically engineered antibody (e.g. diabody) or antibody derived from a transgenic animal. A neutralising antibody may for example be an IgG antibody specific to type II transglutaminase.

A derivative of 2[(2-oxopropyl)thio]imidazolium may for example be 1,2,3,4-tetramethyl-2[(2-oxopropyl)thio]imidazolium chloride, 1,3-dimethyl-4,5-diphenyl-2[(2-oxopropyl)thio]imidazolium trifluoromethylsulfonate. and 1,3-dimethyl-4-phenyl-2[(2-oxopropyl)thio]imidazolium chloride.

Experiments so far undertaken (see 'Experimental' section below) have used cystamine, MDC and putrescine. Other inhibitors of transglutaminase, in particular of type II transglutaminase, may be used to equal effect. The experiments suggest that active site inhibitors such as cystamine are more effective than substrate competitor inhibitors such as MDC and putrescine. Cystamine was found to be more effective than MDC, and both were found to be more effective than putrescine.

Such an inhibitor may be used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

Such an inhibitor may comprise approximately 0.5 - 5 mM MDC or 10 µl - 1 mM cystamine. The volume of solution applied may vary greatly, for example between about 100 µl and 10 ml.

Such an inhibitor may be for use in conjunction with a composition for promoting the healing of wounds with reduced scarring, or for use in conjunction with a composition for promoting the healing of chronic wounds.

Such an inhibitor may be used in a method of treatment or diagnosis of the human or animal body.

Also provided according to the present invention is a method for promoting the healing of wounds or fibrotic disorders with reduced scarring, comprising inhibiting the activity of transglutaminase. The transglutaminase may be type II transglutaminase.

Such a method may comprise the use of an inhibitor of transglutaminase according to the present invention.

The activity of transglutaminase may be inhibited for less than 7 days after wounding. It may be inhibited for less than 120 hours after wounding. It may be inhibited for not more than 60 hours after wounding. It may be inhibited for not more than 48 hours after wounding. It may be inhibited for not more than 36 hours after wounding. It may be inhibited for not more than 24 hours after wounding. The inventor has found that the time and duration of inhibition of the activity of type II transglutaminase also affects the efficacy of treatment - it appears that the profile of members of the TGF-β family present at the wound site varies over time. At early time points there is a higher ratio of TGF-β_1 and TGF-β_2 to TGF-β_3, mostly as a result of platelet degradation. With time the ratio changes as platelet-derived TGF-β_1 decreases and fibroblast-derived TGF-β_3 increases. Therefore the anti-scarring treatment works best if applied at the time of

wounding, and immediately following wounding. By inhibiting the activation of LTGF-β during a particular time period, the relative amounts of fibrotic and non-fibrotic TGF-β at the wound site may be affected. By "amounts of fibrotic and non-fibrotic TGF-β" is meant the quantities of active growth factor.

Such a method may comprise the application or administration of an inhibitor according to the present invention to a site of wounding or fibrosis.

It may comprise the use of an approximately 0.5 - 5 mM dose of MDC. It may comprise the use of an approximately 10 μM - 1 mM dose of cystamine. The dose may be of a volume of approximately 100 μl - 10 ml.

Conversely, the present invention also provides stimulator of transglutaminase activity for use in promoting the healing of chronic wounds. The transglutaminase may be type II transglutaminase.

The stimulator may increase the level of type II transglutaminase at the wound site.

The stimulator may comprise type II transglutaminase or a partially modified form thereof. Partially modified forms include those modified by, for example, addition, deletion or substitution of amino acid residues. For example, substitutions may be conserved. Hence such partially modified molecules may be homologues of type II transglutaminase. They may for example have at least 40% homology with type II transglutaminase. They may for example have at least 50, 60, 70, 80, 90 or 95%

homology with type II transglutaminase.

The stimulator may, for example, increase the level of production of transglutaminase or it may be an inhibitor of transglutaminase breakdown, increasing the half-life of transglutaminase.

Such a stimulator may be used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

Such a stimulator may be used in conjunction with a composition for promoting the healing of wounds with reduced scarring.

Such a stimulator may be for use in a method of treatment or diagnosis of the human or animal body.

Also provided according to the present invention is a method of promoting the healing of chronic wounds comprising stimulating the activity of transglutaminase. It may comprise stimulating the activity of type II transglutaminase.

Such a method may comprise the use of a stimulator of transglutaminase activity according to the present invention.

The activity of transglutaminase may be stimulated for not more than 72 hours after wounding. It may be stimulated for not more than 60 hours after wounding. It may be stimulated for not more than 48 hours after wounding. It may be stimulated for

not more than 36 hours after wounding. It may be stimulated for not more than 24 hours after wounding.

The invention will be further apparent from the following example which shows, by way of example only, inhibitors of transglutaminase for use in promoting the healing of wounds or fibrotic disorder with reduced scarring.

EXPERIMENTAL

Three types of transglutaminase (TGase) inhibitors (MDC, putrescine and cystamine) were tested for their effect upon wound healing. MDC and putrescine are substrate competitors for TGase, and cystamine is an active-site induced inhibitor.

Experimental Model

Chemicals were purchased from Sigma and dissolved in PBS (phosphate buffered saline) to the desired concentrations (below) before injection. All TGase inhibitors and PBS were sterilised using sterile filtration.

Adult male Sprague-Dawley rats weighing 225 - 250 g were anaesthetised by halothane, nitrous oxide and oxygen inhalation. Four full-thickness linear incisions, 1 cm in length, were made on the dorsal skin of the animals using a scalpel. In each animal, two wounds were injected intradermally with TGase inhibitors, one was injected with PBS for sham control and one was unmanipulated for control. Each injection was made to the volume of 100 µl (50 µl in each wound margin). All injections were administered along both wound margins using an insulin syringe.

At days 3, 7 and 8 post-wounding (pw), the animals were killed by chloroform overdose and the wounds were harvested. Each wound was bisected, one half was processed for histology and the other half for immunocytochemistry. In each experiment at least four animals were used per treatment and per dose and per harvest time-point.

Dose Experiments

To determine the optimum dose of TGase inhibitors, three concentrations of each TGase inhibitor were tested in each experiment (Table 1)

Table 1 - Concentrations of TGase inhibitors

Group	MDC	Putrescine	Cystamine
1	50 µM	50 µM	10 µM
2	0.5 mM	0.5 mM	0.1 mM
3	5 mM	5 mM	1 mM

In addition, 1 mM and 2 mM of MDC, and 50 mM and 500 mM of putrescine were also tested separately.

Time and duration of treatment

To determine the optimal time and duration of treatment, three different injection regimes were performed (Table 2)

Table 2 - Time and treatment of TGase inhibitors

Types	Days 0 - 4	Days 0 - 2	Days 5 - 7
MDC	+	+	+
Putrescine	+		
Cystamine		+	+

Since the TGase family consists of several members including plasma TGase (XIII) which catalyses fibrin cross-linking, injection of TGase inhibitors into wounds could also down-regulate this event. By making the fibrin clot looser (i.e. less highly cross-linked) this could facilitate cell infiltration and so result in a more normal restitution of the

dermal architecture (i.e. it might be less scarred). In order to investigate whether TGase inhibitors decrease fibrin cross-linking, another experiment was carried out. In this application, animals were divided into two groups - a first group in which TGase inhibitors were injected into wounds just prior to wounding and a second group in which TGase inhibitors were injected after wounding (when bleeding had stopped). Bleeding times for each wound were measured. All wounds were injected every day for 3 days. The wounds were harvested at days 3 and 80 pw. The wounds harvested at day 3 pw were examined for fibrin clot pattern (below). The wounds harvested at day 80 pw were assessed for scar quality.

Histology and light microscopy

Wounds were fixed in 10 % formal saline and further processed for paraffin embedding and sectioned at 7 µm thickness. Mascon's trichrome staining was used to highlight the major component of ECM (extracellular matrix) - collagen. All slides were examined under a light microscope. The architecture of the wounds was assessed and scored according to the size, density and orientation of newly formed collagen. The wounds harvested at day 3 pw were stained with the MSB method to examine fibrin cross-linking patterns. Photographs were taken using a Leitz Dialux microscope on Kodak 160 ASA Tungsten colour film. At day 80 pw, wounds were photographed by image capture.

Immunocytochemistry

The wounds were embedded in OCT and frozen in liquid nitrogen. Cryosections, 7 µm in thickness, were cut onto poly-L-lysine coated slides and stored at - 20 °C until they were used for staining. All immunostainings were carried out using the biotin-streptavidin amplification technique.

Sections were incubated with the primary antibody for one hour and then washed three times for five minutes with PBS. The sections were then incubated with biotinylated secondary antibody for 40 minutes; washed three times with PBS and finally incubated with FITC (fluorescein-isothiocyanate) conjugated streptavidin for 40 minutes; washed three times with PBS and finally incubated with FITC-conjugated streptavidin for 40 minutes followed by three washes for five minutes with PBS. The sections were mounted on Gelvatol or DARCOL and visualised by epifluorescence microscopy using a Leitz Dialux microscope. Photographs were taken using identical exposure times on Kodak 400 ASA colour film.

The following immunostaining was carried out:

1. Macrophages and monocytes: monoclonal mouse anti-rat macrophages and monocytes (ED1, Serotec, Oxford, UK) was used to observe inflammatory cells.

2. Fibronectin: polyclonal rabbit anti-rat plasma fibronectin (Chemicon Int. Inc., Temceula, CA, USA) was used to examine fibronectin.

3. von Willebrand factor: Polyclonal rabbit anti-rat human plasma von Willebrand factor (Dakopatts. Glostrup, Denmark) was used to study new blood vessel formation (angiogenesis).

TGase assay

A polyclonal rabbit antibody against guinea pig tissue TGase was diluted 1 in 20 and cryosections from normal wounds 1, 3, 6, and 24 hours and 3, 4, 7, 14 and 40 days pw were used for immunostaining to localise the expression and distribution of TGase in the wounds. Normal skin tissue was used for comparison. Procedures for streptavidin-biotin amplification immunostaining were followed as above. Non-specific binding was

blocked with 1% BSA/TBS solution.

Detection of active and latent TGF-β

To determine the mechanism of TGase inhibitors on wound healing, an antibody to total TGF-β and a truncated TGF-β type II receptor (RIIX) for active TGF-β were used in dual immunostaining to detect the relative amounts of active and latent TGF-β in the wound. Chicken anti-human TGF-β antibody (AB-101-NA, R&D Systems) can bind both active and latent TGF-β. RIIX can only bind to active TGF-β which was purified from RIIX transformed *E. coli* cells and labelled with FITC using a standard protocol. AB-101-NA was detected by TRITC labelled rabbit anti-chicken secondary antibody (T6903, Sigma). Cryosections from days 3 and 7 pw were tested in this assay. Slides were visualised and photographed by double exposure using the FITC and TRITC filters.

Kinetics

In order to determine how long MDC lasts in a wound, wounds were treated with a single injection of 1 mM MDC at the time of wounding. Control wounds were injected with PBS. Five rats were used in the first experiment. The wounds were excised at 1, 3, 4, 24 and 48 hours pw. All of the wounds were embedded in OCT. Cryosections were examined for the fluorescence of MDC using a fluorescence microscope. The fluorescence was rather weak and became invisible three hours after injection. A repeat experiment was performed using 5 mM MDC, examination timing being set at 0, 1, 3, 6 and 24 hours pw. Bright fluorescence was observed at 0 hours pw, the fluorescence lasting until 6 hours pw. The distribution of transglutaminase in the connective tissue was observed very clearly, a lot of fluorescence-stained fibres being observed in the ECM.

Results

Macroscopic appearance

The majority (70%) of MDC and cystamine-treated wounds healed with a fine linear scar which was hardly discernable at 80 days pw. 0.5 mM and 5 mM of MDC and 0.1 mM of cystamine had the best anti-scarring effect. By contrast, control and sham-control wounds healed with an obvious scar. Putrescine had no significant effect upon scarring at 0.5 mM or less and at 5 mM or higher it has a toxic effect, demonstrated by skin necrosis in the wound area.

Histological analysis

Although all of the wounds were macroscopically equally healed at day 7 pw, the histological appearance was different between the treated and control wounds. The wound area of MDC and cystamine-treated wounds was narrower than that of the control wounds. The amount of collagen in the treated wounds, both in the treated margin and in the centre of the wounds, was greatly decreased compared to the control wounds. This effect appeared dose-dependent. 50 μM of MDC did not have an obvious effect on the total amount of collagen.

At 80 days pw, the wounds treated with MDC and cystamine for both three and five days healed with a better scar than the control wounds. However, the wounds which received TGase inhibitors at 5-7 days appeared no different from control wounds. The architecture of the neodermis of the treated wounds was markedly improved when compared to the controls. Newly formed collagen was organised in a "basket-weave" pattern, appearing similar to normal dermis but unlike the control wounds in which collagen fibres were thinner and more densely packed, lying parallel to the wound surface. The scores of the

wound architecture are shown in Table 3 (treatment duration for MDC is 5 days; cystamine treatment is 3 days).

Table 3- Dermal architecture

Group	MDC	cystamine	PBS	control
50/10* μM	3	2	3	4
0.5/0.1* mM	2	1	4	4
5/1* mM	2	2	4	4

* indicates cystamine concentrations.

Scale: 0 - same as normal dermis

4 - obvious scar tissue

The scale is linear, scoring reflecting the density, spacing and orientation of collagen fibres and their diameter in the wound site compared to the surrounding normal dermis.

It can be seen that 0.5 mM and 5 mM of MDC and the three concentrations of cystamine had a good anti-scarring effect. There was no obvious difference between the control and sham control wounds.

The cross-linking of the fibrin clot in treated wounds was decreased and appeared less compact than that found in the control wounds, irrespective of whether the wounds received TGase inhibitors pre- or post-wounding.

Comparison of bleeding times of the treated wounds with the control wounds showed that the bleeding times of some wounds treated with the high dose of TGase inhibitors

were 1-2 minutes longer than the control wounds (which had a bleeding time of 15 minutes).

Inflammatory response

The number of ED1 positive cells in the wound treated with MDC and cystamine appeared little different to that of the control wounds at day 3 pw. It was found that there were more ED1 positive cells at the injection site of 5 mM MDC at this time point, but that these cells were no longer present at day 7 pw. At day 7 pw, the number of inflammatory cells in treated wounds was reduced compared to the number in the control wounds.

The data indicates that TGF-β activation may be down-regulated by TGase inhibitors. However, inhibitors themselves might elicit a transient inflammatory response at high concentrations in the first four days post-wounding. This is a surprising result as normally anti-scarring agents decrease inflammation, or agents which increase inflammation are not anti-scarring. This suggests that TGase inhibitors may be acting either by preventing activation of TGF-β_1 for some time post-wounding and/or by interfering with the structure of the fibrin clot.

Fibronectin

The treated wounds showed a marked decrease in the intensity of staining for fibronectin at days 3 and 7 pw compared to the control wounds.

Fibronectin is a major component of the extracellular matrix produced by fibroblasts at the early stages of wound repair, which plays an important role in matrix organisation.

It facilitates the adhesion of connective tissue cells and other extracellular matrix molecules simultaneously and may serve as a template for collagen deposition. TGF-β_1 is a powerful stimulant of fibroblast fibronectin production. Reduced fibronectin in the treated wounds may be due to inhibition of TGF-β_1 activity by TGase inhibitors.

Angiogenesis

There was no obvious difference between the treated and control wounds at days 3 and 7 pw.

Angiogenesis is regulated by several factors simultaneously, such as VEGF and FGF as well as TGF-$\beta_{1,2}$. TGF-β functions on angiogenesis indirectly, by the recruitment of more macrophages to the wound site, which in turn secrete angiogenic molecules. Although TGF-β activity was found to be down-regulated by TGase inhibitors, macrophage numbers in the wounds were not dramatically decreased, possibly due to the pro-inflammatory effect of TGase inhibitors. The angiogenic cytokines produced by these inflammatory cells in the wounds may stimulate blood vessel formation which could balance the effect of the reduction of TGF-β_1 activity by TGase inhibitors.

TGase expression and distribution

In the normal skin, TGase was absent or very weakly localised in the epidermis, hair follicles and muscle tissue. After wounding, TGase was localised to the fibrin clot, sebaceous glands and endothelial cells as well as epithelial cells and hair follicles from 6 hours to 7 days pw. TGase stained with the greatest intensity at the wound margins during this time period, with the highest intensity from 3 to 5 days pw. After 7 days pw, TGase staining became much weaker, and staining disappeared thereafter.

TGF-β activation

The activation of TGF-β in the treated wounds appeared to be down-regulated as shown by decreased RIIX staining in the double stained wounds at day 3 pw, but this down-regulation was not obvious at day 7 pw. This may be due to rapid clearance of TGase inhibitors from the wounds.

Conclusions

Hence TGase inhibitors. in particular cystamine and MDC. have an anti-scarring effect upon wounds.

The optimum dose of MDC and cystamine is 0.1 - 1 mM, and early administration of TGase inhibitors appears to be necessary in order to optimise wound healing, inhibiting the autocatalytic and autoinductive cascade of TGF-β amplification at the wound site.

MDC and cystamine have a better anti-scarring effect than putrescine. and cystamine has a better anti-scarring effect than MDC. 0.1 mM cystamine has the same anti-scarring effect as 0.5 mM MDC, but has no pro-inflammatory effects at any concentration tested. Additionally, because cystamine is an active-site induced TGase inhibitor, its inhibitory effect is irreversible. In contrast, MDC is a substrate competitor and its inhibitory function is reversible, depending upon its concentration relative to that of the natural substrate. In addition, MDC appears to be cleared from the injection site very quickly (approximately 6 hours after injection).

Putrescine belongs to the same class of inhibitor as MDC, but it has a less potent

inhibitory effect then MDC. It needs to be given at higher doses to inhibit TGase. However, toxic effects occur at concentrations of 5 mM and higher.

CLAIMS

1. An inhibitor of transglutaminase for use in promoting the healing of wounds or fibrotic disorders with reduced scarring.

2. An inhibitor of transglutaminase according to claim 1, the transglutaminase being type II transglutaminase.

3. An inhibitor of transglutaminase according to either one of claims 1 or 2, the inhibitor being an active site inhibitor or a substrate competitive inhibitor.

4. An inhibitor according to any one of the preceding claims, the inhibitor being selected from any one of the group of cystamine, monodansylcadaverine, putrescine, bacitracin, 2-[3-(diallylamino)propionyl]benzothiophene, a neutralising antibody or an antigen binding fragment thereof specific to transglutaminase and a derivative of 2[(2-oxopropyl) thio] imidazolium.

5. An inhibitor according to claim 4, the neutralising antibody being an IgG antibody specific to transglutaminase.

6. A composition according to claim 4, the neutralising antibody being selected from any one of the group of a monoclonal antibody, a polyclonal antibody, and a genetically engineered antibody.

7. An inhibitor according to any one of claims 4-6, the antibody being either

a phage-derived antibody or an antibody derived from a transgenic mouse.

8. An inhibitor according to claim 4, the derivative of 2[(2-oxopropyl)thio]imidazolium being selected from any one of the group of 1,2,3,4-tetramethyl-2[(2-oxopropyl)thio]imidazolium chloride, 1,3-dimethyl-4.5-diphenyl-2[(2-oxopropyl) thio] imidazolium trifluoromethylsulfonate, and 1,3-dimethyl-4-phenyl-2[(2-oxopropyl)thio]imidazolium chloride.

9. An inhibitor according to any one of the preceding claims for use in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

10. An inhibitor according to any one of the preceding claims for use in conjunction with a composition for promoting the healing of wounds with reduced scarring.

11. An inhibitor according to any one of the preceding claims for use in conjunction with a composition for promoting the healing of chronic wounds.

12. A method for promoting the healing of wounds or fibrotic disorders with reduced scarring comprising inhibiting the activity of transglutaminase.

13. A method for promoting the healing of wounds or fibrotic disorders with reduced scarring according to claim 12 comprising inhibiting the activity of type II transglutaminase.

14. A method according to either one of claims 12 or 13 comprising the use of a composition according to any one of claims 1-11.

15. A method according to any one of claims 12-14 comprising inhibiting the activity of transglutaminase for less than seven days after wounding or onset.

16. A method according to any of claims 12-15 comprising the use of an approximately 0.5 - 5 mM dose of MDC.

17. A method according to any one of claims 12-15 comprising the use of an approximately 10 μM - 1 mM dose of cystamine.

18. A method according to either one of claims 16 and 17, the dose being of a volume of approximately 100 μl - 10 ml.

19. A stimulator of transglutaminase activity for use in promoting the healing of chronic wounds.

20. A stimulator of transglutaminase activity according to claim 19, the transglutaminase being type II transglutaminase.

21. A stimulator of transglutaminase activity according to claim 16 comprising type II transglutaminase or a partially modified form thereof.

22. A stimulator of transglutaminase activity according to either one of claims

19 or 20 comprising either a stimulator of type II transglutaminase production or an inhibitor of type II transglutaminase breakdown.

23. A stimulator of transglutaminase activity according to any one of claims 19-22 for use in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

24. A stimulator of transglutaminase activity according to any one of claims 19-23 used in conjunction with a composition for promoting the healing of chronic wounds.

25. A stimulator of transglutaminase activity according to any one of claims 19-24 for use in conjunction with a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

26. A method for promoting the healing of chronic wounds comprising stimulating the activity of transglutaminase.

27. A method for promoting the healing of chronic wounds according to claim 26, the transglutaminase being type II transglutaminase.

28. A method according to either one of claims 26 or 27, comprising the use of a stimulator of transglutaminase according to any one of claims 19-25.

24. A method according to any one of claims 26-28 comprising stimulating the

activity of type II transglutaminase for not more than 72 hours after wounding.

INTERNATIONAL SEARCH REPORT

Inte· ·nal Application No
PC1/GB 96/01785

A. CLASSIFICATION F SUBJECT MATTER
IPC 6 A61K31/00 A61K31/13 A61K31/145 A61K31/18 A61K31/40
A61K38/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 18760 A (UNIVERSITY OF MANITOBA) 30 September 1993 see the whole document ---	1-4,9, 12-14
P,X	PLAST. RECONSTR. SURG., vol. 97, no. 1, 1996, pages 117-123, XP000609865 K.N. DOLYNCHUK ET AL.: "Topical putrescine (fibrostat) in treatment of hypertrophic scars: phase II study." see the whole document ---	1-4,9, 12-14
X	EP 0 598 133 A (AJINOMOTO CO INC.) 25 May 1994 see the whole document ---	19, 23-26,28
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
11 February 1997	26.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Klaver, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte· ·nal Application No
PC1/GB 96/01785

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOCHIM. BIOPHYS. ACTA., vol. 762, no. 3, 1983, pages 414-419, XP000609891 M.M. CORNWELL ET AL.: "Inhibition of the adhesion of chinese hamster ovary cells by the naphthylsulfonamides dansylcadaverine and n-(6-aminohexyl)-5-chloro-naphthylenesulfonamide (W7)." ---	
A	EP 0 411 908 A (MERCK & CO.) 6 February 1991 ---	
A	WO 91 10427 A (UNIVERSITY OF TEXAS) 25 July 1991 -----	

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 96/01785

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 13-18 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1) Claims 1-18: Use of a transglutaminase inhibitor to promote wound healing or to treat fibrotic disorders with reduced scarring.
2) Claims 19-24: Use of a transglutaminase stimulator to promote the healing of chronic wounds.

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PC1/GB 96/01785

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9318760	30-09-93	AU-A-	1544892	21-10-93
		BR-A-	9207109	12-12-95
		EP-A-	0632723	11-01-95
		JP-T-	7504154	11-05-95
EP-A-598133	25-05-94	US-A-	5525335	11-06-96
		WO-A-	9320837	28-10-93
EP-A-411908	06-02-91	US-A-	5021440	04-06-91
		CA-A-	2022114	01-02-91
		JP-A-	3128360	31-05-91
WO-A-9110427	25-07-91	US-A-	5124358	23-06-92
		AU-A-	7478991	05-08-91